Improving Integrated Pest Management of Spider Mites on Almond

Project No.: **16-ENTO17-Tollerup**

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Objectives:

1. Determine the effectiveness of a prophylactic, early-season application of abamectin to manage spider mite populations on almond.
2. Determine if southern San Joaquin Valley populations of *Tetranychus pacificus* and *Tetranychus urticae* have developed resistance to abamectin.
   a. Establish base-line LD_{50} for susceptible strains of Pacific and twospotted spider mites to abamectin.
   b. Evaluate spider mite populations collected from locations in the southern and central San Joaquin Valley against the susceptible strain.

Interpretive Summary:

We conducted a large-scale field experiment in 2016 and 2017 to evaluate the effectiveness of abamectin applied as a prophylactic treatment against spider mites. Results from both years indicated that the preventative treatment did not provide any detectible benefit. In 2017 we monitored plots weekly from May to September employing the spider mite sampling method developed by the University of California, Statewide IPM Program. Mean mite density (motile stages plus eggs) per leaf remained well below the UC treatment threshold of two mites per leaf until mid-July (**Figure 1**) – approximately two weeks after the initiation of hull split. This is a period when miticides are typically not applied due to the proximity of harvest.

Statistical analyses of mite density data: motile stages plus eggs (meanTot), motile stages (meanMS), and eggs (meanEgg) were conducted for each of five sample dates from July 24th to August 21st (**Tables 1 to 3**). In the abamectin prophylactic – treated plots, meanTot densities tended to have numerically lower means than all other treatments across sample dates. However, substantial statistical overlap occurred among the treatments, and no definitive benefit of abamectin applied prophylactically could be established.

Approximately one week after meanTot densities began to increase, populations of the spider mite predator, sixspotted thrips, also began to increase (**Figures 1 and 2**). Over only a 3-week period, the predator thrips facilitated a precipitous reduction of spider mite motile stages

and eggs from a peak of ~13 per leaf to undetectable levels in all plots by late August (**Figure 1**).

The LD_{50} of susceptible *T. pacificus* and *T. urticae* populations (**Table 4**) were 0.39 and 0.38 ppm respectively (**Table 5**). I evaluated *T. pacificus* mite populations from eight commercially-managed almond orchards from within Fresno, Kern, Kings, and Tulare counties (**Table 4**) between early June and September of 2017. To date, successful probit analyses have been conducted on mortality data from five of eight orchard sample sites (**Table 4**).

Probit analyses indicated an LD_{50} ppm between 1.16 (FRSCO1) and 7.23 (FRSCO4). Although these values do not exceed the Agri-Mek SC recommended field rates of 11.5 – 23 ppm, they are 3 and 18.5-fold more resistant than the susceptible population. Anecdotal evidence from growers and pest control advisers (PCAs) suggest that spider mites are “more difficult” to manage with abamectin than in previous years. These results support those observations. During 2018, I plan on continuing this study and evaluate spider mite populations in the northern San Joaquin Valley and the Sacramento Valley region.

Materials and Methods:

<u>Objective 1. Effectiveness of Prophylactic Abamectin Applications.</u> This work first began in 2015 at Wonderful Orchards as an unfunded project. In 2016, 2017, as well as 2018, Wonderful Orchards provided experimental plots, technical support, and staff to collect and process samples. We established 30 plots at two, 150-acre almond blocks at Wonderful Orchards (sites 1 and 2) near Shafter, Kern County. Five treatments: 1) untreated control, 2) abamectin applied at the UC recommended treatment threshold of 2 mites/leaf, 3) abamectin applied as a prophylactic, 4) Nealta, applied as a prophylactic, and 5) Zeal, applied as a prophylactic were randomly assigned to plots. Each treatment was replicated six times (3 at each site). Wonderful Orchards applied treatments using a commercial air-blast sprayer at 100 gallons per acre. Spider mite populations were assessed weekly from May to early September (2017) using the University of California, Statewide IPM Program sampling recommendation for spider mites on almond (Zalom et al. 2015). Sixspotted thrips, *Scolothrips sexmaculatus* (Pergande), was monitored using 3.5” x 8”, blue/yellow sticky cards (Alpha Scents, West Linn, OR). A card was placed at approximately six feet above the soil surface in the canopy of a tree. Two plots of each treatment were monitored, and cards were changed weekly or bi-weekly from May to early September (2017).

In 2017, leaf samples were collected from seven trees per plot, one tree in each of seven areas consisting of 25 trees (5 rows x 5 trees). In each plot, sample areas were located at the center and three spaced along each of two of the plot’s edges. Fifteen leaves from each sample tree were collected and placed in labeled plastic bags and stored at approximately 45°F in the laboratory until processed. Mite motile stages and eggs were counted using a binocular microscope and the mean number per leaf was calculated for each sample tree.

<u>Statistical Analyses.</u> For 2017 data, I utilized a general linear model (Proc GLM) based on the SAS statistical software (SAS Institute 2011). Over all sample dates, the treatment effect and the treatment x site effect were evaluated. I employed the least squares means option (lsmeans) to determine differences among treatment means of motile stages plus eggs

(meanTot), motile stages only (meanMS), and eggs only (meanEgg) over five sample dates from July 24th to August 21st.

Objective 2. Establishing Colony to Determine Base-Line LD_{50} of Susceptible Spider Mite Strains. We established susceptibility baseline data for two spider mite species, *Tetranychus pacificus* McGregor and *Tetranychus urticae* Koch using laboratory colonies unexposed to miticides. A colony of *T. pacificus* was established in Kris Tollerup's laboratory using individuals obtained from a population maintained in the Frank Zalom laboratory at the University of California, Davis on bean, *Phaseolus vulgarus* (L.). The colony of *T. pacificus* was maintained at the University of California, Kearney Agricultural Research and Extension Center (KARE) in Parlier also on *P. vulgarus* and under similar laboratory conditions. To reduce the probability of cross contamination of susceptible spider mite species, a colony of *T. urticae* was kept at UC Davis in the Zalom laboratory and maintained by an employee of Kris Tollerup's laboratory.

Collection of Field Populations. Between mid-May and early June, I monitored 10 to 15 almond orchards located in southern and mid-Fresno county, and northern Kern County for spider mite populations at a density necessary to evaluate with bioassays. Spider mite infestations began occurring at early June and I sampled from eight commercial orchard sites between 4 June to September 2017. Mite-infested leaves were collected by cutting branches approximately 10 mm in diameter from the interior area of five to 10 trees. Excised branches were placed in plastic bags and kept in an ice chest until arriving to the laboratory. Sampled mites were maintained in the laboratory on the sampled branches and were kept for no longer than 72 h before conducting bioassays.

Laboratory Bioassays. For each bioassay experiment, we evaluated four concentrations of abamectin (Agri-Mek SC, 8% ai [ai wt/v], Syngenta Crop Protection, LLC, Greensboro, NC) in water plus an untreated control. The concentrations evaluated were estimated to kill ~95% of the test population at the highest level and ~10% at the lowest. A stock solution (65.5 ppm) was prepared and concentrations were prepared using serial dilutions.

A replicate consisted of 10 mature females placed on an 18-mm leaf disc cut from a mature almond leaf. On the same date as a bioassay experiment, almond leaves were collected from an orchard not treated with miticides located at KARE. Leaves were collected, washed, and cut into discs. Leaf discs were placed on paper towels and abamectin dilutions applied using a PREVAL® sprayer (Chicago Aerosol, Coal City, IL), until the surface of the disc was completely covered. Discs were allowed to dry for approximately one hour then placed on moist cotton contained in a 150 x 15 mm plastic Petri dish arena according to treatment.

Ten adult females were placed on each leaf disc using a small camel-hair paint brush. Arenas were kept in a designated area of a laboratory work space and kept at 72 °F and 30% RH. Mite mortality was scored at 24 h; mites were considered dead if unable to ambulate.

Statistical Analysis Probit analysis based on SAS software (SAS Institute 2011) was used. The analyses determined the lethal concentration, LC_{50}, with respective 95% confidence limits. Resistant ratios were calculated for each population by dividing the LC_{50} value for field populations by the LC_{50} value of the susceptible population.

Results and Discussion:

Objective 1. Effectiveness of Prophylactic Abamectin Applications. Mean mite densities in this 2017 study did not begin reaching threshold levels across all treatments until late July (**Figure 1**). And therefore, due to the relatively late arrival of spider mites, treatment 2 (abamectin @ threshold) was not applied. Spider mite populations among all the plots occurred only over a period of approximately five weeks from mid-July to late August. Analysis of (treatment x site) interaction over that period indicated that no significant interaction occurred (F =0.58; df = 2; P = 0.5627) therefore data were pooled across sites.

Analyses of meanTot densities on the five sample dates from July 24th to August 21st indicated no significant benefit of the abamectin prophylactic treatment. Mean densities of abamectin prophylactic-treated plots were numerically lower on three (July 31st and August 7th and 14th) of the five sample dates. However, no definitive statistical evidence supporting the treatment emerged due to considerable statistical overlap (**Table 1**).

Similar to meanTot, meanMS and meanEgg were numerically lower on the same three dates, July 31st and August 7th and 14th (**Tables 2 and 3**). Statistical analysis of meanSM and meanEgg indicated that no significant interaction (F = 0.77; df = 4; P = 0.543) between life stages i.e. motile or egg occurred. In other words, prophylactic applications of abamectin, as well as Nealta or Zeal did not differentially affect the motile or egg stage.

Peak densities among treatments did not occur until early to mid-August. This is the period well into Nonpareil harvest; irrigation is reduced, and trees become stressed and commonly succumb to mite infestation. Moreover, producers typically forgo miticide applications at that period due to the proximity of harvest.

Sixspotted thrips (SST), *Scolothrips sexmaculatus* (Pergande) is an effective predator of tetranychid mites as well as several other mite pests and commonly occurs in North America (Bailey 1936). The species is an effective predator at high ambient temperatures making it well suited for California's almond production regions. Coville and Allen (1977) reported that at 86° F, *S. sexmaculatus* requires only 2.7 days to double in population. And moreover, a single female can consume just under 50 spider mite eggs per day.

The SST population we monitored during 2017 effectively reduced spider mite motile stages and eggs and our data shows the importance of the predator, especially in managing spider mites during late-season. The thrips was present, in all experimental plots during early May; then dropped to below detectable levels by early June. The early SST population fed on spider mites and eggs than decreased sharply due to the lack of prey. Approximately one week after meanTot densities began to increase, populations of SST also began to increase (**Figures 1 and 2**). Between August 7th and August 28th, the predator thrips facilitated a precipitous reduction of motile stages and eggs from peaks of (meanTot ± SE) 11.79 ± 2.45 (untreated control) and 13.25 ± 2.62 (abamectin @ threshold, not treated) to undetectable levels in all plots (**Figures 1 and 2**).

Objective 2. Establishing Base-Line LD_{50} of Susceptible Spider Mites. To establish an LD_{50} of abamectin for susceptible populations of *T. pacificus* and *T. urticae* approximately 455 and 593 individual mites were used respectively. Abamectin concentrations of: 0.23, 0.33, 1.84, and 2.4 ppm were used to evaluate *T. pacificus* and an LD_{50} (95% CL) of 0.39 (0.27 – 0.52) was determined (**Table 5**). An LD_{50} of 0.38 (0.30 – 0.49) ppm was established for the susceptible population of *T. urticae* using concentrations of: 0.145, 0.21, 0.88, and 2.67 ppm (**Table 5**).

Tetranychus pacificus mite populations were sampled from seven almond orchards located in Fresno, Kern, Kings, and Tulare counties (**Table 4**) and a single orchard at the University of California, West Side Research and Extension Center in Fresno County between early June and late September of 2017.

The model effect for the probit analyses was significant for five of the orchard sample sites, FRSCO1, and FRSCO3, and FRSCO4, FRSCO5 and TULCO1 (**Table 5**). The susceptibility of field-collected *T. pacificus* varied considerably with FRSCO1 having a ~3-fold and FRSCO4 having an 18.5-fold greater tolerance than the susceptible population (**Table 5**).

Ratio values calculated in this study indicate resistance in field populations of *T. pacificus*, however the values are not exceptionally large. In *T. urticae*, for example, Ferreira et al. (2015) estimated resistance ratios of 8.0 to 295,270 in populations collected in Brazil from cotton and ornamental flower plantations. On ornamental nursery plants produced in California, Campos et al.(1995) reported resistance ratios for *T. urticae* from 5 to 87. Also, Zalom evaluated populations of *T. urticae* on strawberry from Orange, Ventura, Santa Barbara, San Luis Obispo, and Monterey counties and found populations between 125 and 1,738-fold more resistant than a susceptible population.

The LD_{50} ppm values I determined in this study (**Table 5**) did not exceed the Agri-Mek SC recommended field rate of 1.75 - 3.5 fl. oz/Ac (4.4 – 8.7 g AI/Ac or 11.5 – 23 ppm). Albeit, results from this study support observations by PCAs and growers that populations of *T. pacificus* as well as *T. urticae* on almond have become more difficult to manage with abamectin than in past years.

Abamectin resistance is unstable in the absence of selection (Stumpf and Nauen 2001, Sato et al. 2005). For instance, Brown et al. (2017), found that on cotton large shifts in *T. urticae* susceptibility occurred from year to year. They concluded that enough time elapses between seasons that susceptibility can reestablish. This characteristic of abamectin is beneficial in spider mite management strategies (Dennehy et al. 1990). Given the low level of resistance I found during this study, abamectin should remain an effective tool if growers and PCAs take resistance management seriously.

Conclusion: In this study, I showed that a moderate level of resistance to abamectin has developed in some Pacific mite populations in the south and mid-San Joaquin Valley. Abamectin is an important tool for managing spider mites. To maintain the miticide's effectiveness, the almond industry should work to eliminate the practice of making prophylactic applications and rely more on monitoring during May for making early-season treatment decisions. Moreover, the industry must work toward a greater reliance on sixspotted thrips.

The predator plays a role in managing early-season mite populations and as results of this study showed, play a critical role in managing spider mites at late-season.

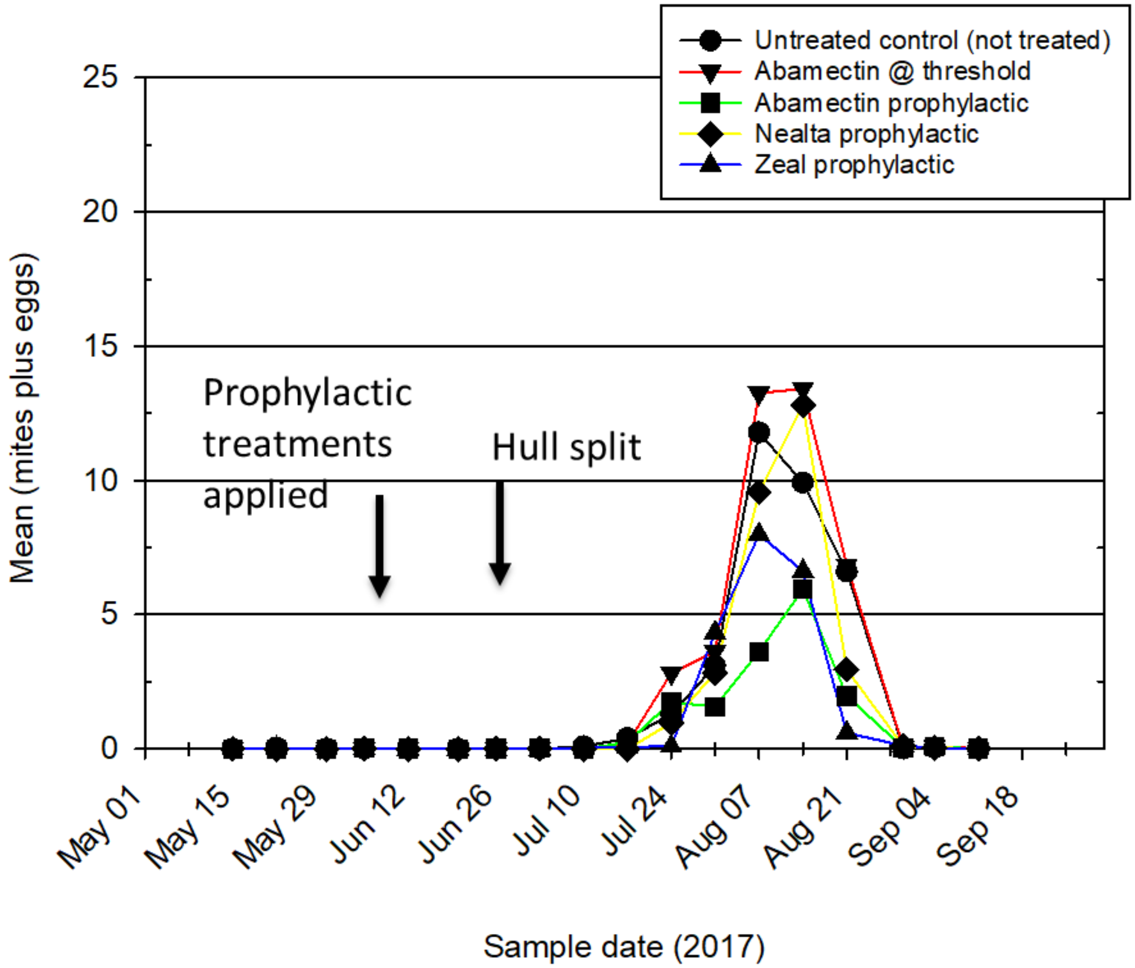

Figure 1. Mean density per leaf of spider mites plus eggs on almond. Data pooled from two sites located near Shafter, Kern County.

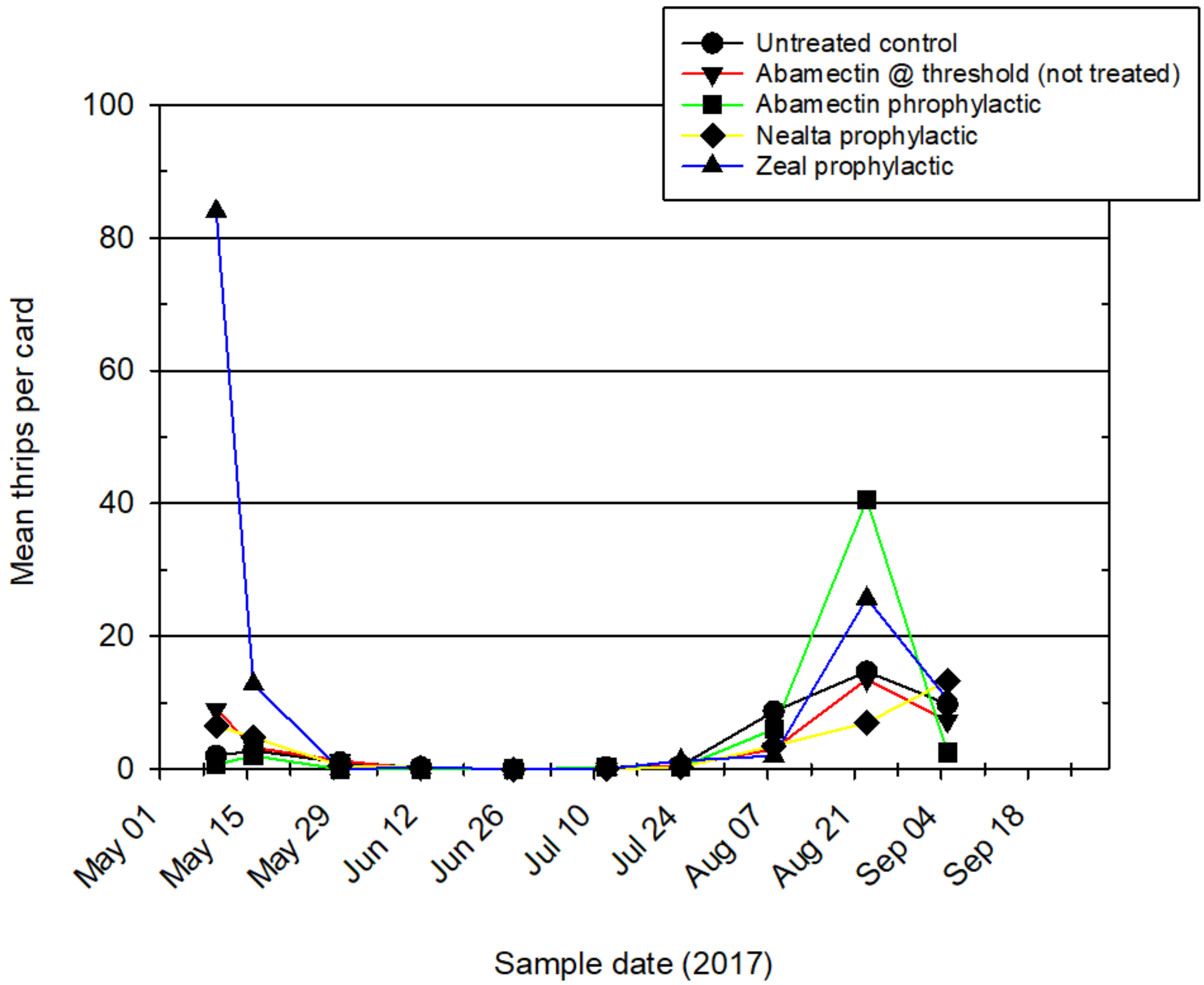

Figure 2. Mean sixspotted thrips density per yellow sticky card. Data were pooled from two sites located near Shafter, Kern County.

Table 1. Mean number of mites per leaf (motile stages plus eggs) on almond treated with various miticides in 2017**.**

Date 2017	Untreated Control	Abamectin @ Threshold (not applied)	Abamectin Prophylactic	Nealta Prophylactic	Zeal Prophylactic
7/24	1.3 ± 0.3 ab[1]	2.82 ± 0.99 a	1.75 ± 0.96 a	0.96 ± 0.37 ab	0.1 ± 0.06 b
7/31	3.09 ± 0.75 b	3.62 ± 0.73 ab	1.57 ± 0.53 b	2.84 ± 0.93 ab	4.33 ± 1.07 a
8/7	11.79 ± 2.45 ab	13.25 ± 2.62 a	3.62 ± 0.84 c	9.56 ± 3.09 ab	7.98 ± 1.53 bc
8/14	9.91 ± 1.74 b	13.42 ± 2.61 a	5.94 ± 0.95 c	12.81 ± 2.09 ab	6.62 ± 1.42 c
8/21	6.6 ± 1.5 a	6.84 ± 1.42 a	1.96 ± 0.68 bc	2.95 ± 0.91 b	0.6 ± 0.23 c

[1]Means within a row followed by a different letter are significantly different. ($P < 0.05$; LS-means, pairwise, *t*-test).

Table 2. Mean number of motile mites per leaf on almond treated with various miticides in 2017.

	Mean spider mites (motile stages) **± SE**				
Date 2017	Untreated Control	Abamectin @ Threshold (not applied)	Abamectin Prophylactic	Nealta Prophylactic	Zeal Prophylactic
7/24	0.67 ± 0.17 bc[1]	0.82 ± 0.28 ab	0.75 ± 0.37 a	0.38 ± 0.13 bc	0.03 ± 0.02 c
7/31	1.18 ± 0.31	1.24 ± 0.25	0.58 ± 0.18	1.12 ± 0.41	1.4 ± 0.36
8/7	7.27 ± 1.72 abc	7.99 ± 1.63 a	2.38 ± 0.65 c	5.87 ± 2.1 ab	5 ± 0.96 c
8/14	5.31 ± 0.93 bc	8.7 ± 2.04 a	3.57 ± 0.64 c	6.94 ± 1.11 b	3.86 ± 0.87 c
8/21	4.27 ± 0.96 a	4.67 ± 1.01 a	1.05 ± 0.48 b	1.55 ± 0.52 b	0.32 ± 0.1 b

[1]Means within a row followed by a different letter are significantly different. ($P < 0.05$; LS-means, pairwise, *t*-test).

Table 3. Mean number of mite eggs per leaf on almond treated with various miticides in 2017.

	Mean spider mite eggs **± SE**				
Date 2017	Untreated Control	Abamectin @ Threshold (not applied)	Abamectin Prophylactic	Nealta Prophylactic	Zeal Prophylactic
7/24	0.64 ± 0.15 ab[1]	2 ± 0.72 a	1.01 ± 0.59 a	0.58 ± 0.27 ab	0.06 ± 0.04 b
7/31	1.92 ± 0.46 b	2.38 ± 0.5 ab	1 ± 0.36 b	1.71 ± 0.54 b	2.93 ± 0.73 a
8/7	4.52 ± 0.87 ab	5.26 ± 1.1 a	1.24 ± 0.27 c	3.7 ± 1.06 ab	2.97 ± 0.64 bc
8/14	4.6 ± 0.94 a	4.72 ± 0.74 a	2.36 ± 0.42 b	5.88 ± 1.06 a	2.75 ± 0.65 b
8/21	2.33 ± 0.71 a	2.17 ± 0.61 a	0.92 ± 0.24 bc	1.4 ± 0.44 b	0.28 ± 0.14 c

[1]Means within a row followed by a different letter are significantly different. ($P < 0.05$; LS-means, pairwise, *t*-test).

Table 4. Code and location of spider mite sample sites in 2017.

	Location	Species
SUS1	University of California, Kearney Ag Station	*T. pacificus*
SUS2	University of California, Davis, Zalom laboratory	*T. urticae*
FRSCO1	Navelencia, Fresno Co	*T. pacificus*
FRSCO2	Fresno, Fresno Co	*T. pacificus*
FRSCO3	Raisin City, Fresno Co	*T. pacificus*
FRSCO4	University of California, Westside Field Station	*T. pacificus*
FRSCO5	West Manning Ave	*T. pacificus*
KERCO1	McFarland, Kern Co	*T. pacificus*
KERCO2	Schofield, Kern Co	*T. pacificus*
TULCO1	Corcoran, Tulare Co	*T. pacificus*

Table 5. Concentration response to abamectin of susceptible and field-collected populations of *Tetranychus pacificus* in 2017.

Population	Slope (SEM)	X^2	LC_{50} (95% CL), ppm	Resistance ratio
SUS1	1.33 (0.17)	59.8	0.39 (0.27 - 0.52)	-
SUS2	1.38 (0.15)	90.6	0.38 (0.30 - 0.49	-
FRSCO1	1.50 (0.16)	84.1	1.16 (0.98 - 1.14)	3
FRSCO2		0.15		
FRSCO3	0.72 (0.14)	27.9	6.24 (3.63 - 12.77)	16
FRSCO4	1.07 (0.18)	33.8	7.23 (4.7 – 14.26)	18.5
FRSCO5	0.81 (0.12)	42.2	2.28 (1.51 – 3.55)	5.8
KERCO1		0.59		
KERCO2		1.68		
TULCO1	0.53 (0.21)	6.71	5.11 (1.83 - 2375)	13.1

Research Effort Recent Publications:

None currently.

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